J Gen Plant Pathol (2006) 72:323–327
DOI 10.1007/s10327-006-0291-y

FUNGAL DISEASES

Short communication

Daisuke Yamagishi · Hajime Akamatsu · Hiroshi Otani
Motoichiro Kodama

Pathological evaluation of host-specific AAL-toxins and fumonisin mycotoxins produced by *Alternaria* and *Fusarium* species

Received: October 28, 2005 / Accepted: February 6, 2006

Abstract Host-specific AAL-toxins and mycotoxin fumonisins are structurally related and were originally isolated from the tomato pathotype of *Alternaria alternata* and from *Fusarium verticillioides,* respectively. Previous reports on the production of fumonisin derivatives by the tomato pathotype suggested a possible involvement in the pathogenicity of the pathogen. Here, we have evaluated the role of fumonisin in *A. alternata*–tomato interactions. The results indicate that highly pathogenic isolates of *A. alternata* tomato pathotype produce AAL-toxin as the sole toxin, strongly implicating it as a pathogenicity factor. The related compound, fumonisin, is also toxigenic and has infection-inducing activity on susceptible tomato plants.

Key words *Alternaria alternata* tomato pathotype · *Fusarium* · Tomato · AAL-toxin · Fumonisin · Pathogenicity

Phytotoxins and mycotoxins produced by fungal plant pathogens are generally low molecular weight secondary metabolites that exert toxic effects on host plants and animals, respectively. Among these phytotoxins, host-specific toxins (HSTs) are critical determinants of pathogenicity or virulence in several plant–pathogen interactions (Otani et al. 1995). *Alternaria* species are known to produce HSTs such as AAL-, ACR-, ACT-, AF-, AK-, AM-, AT-toxins (Otani et al. 1995). On the other hand, *Fusarium* (*Gibberella*) species produce many kinds of mycotoxins such as fumonisin, moniliformin, and fusaric acid (Fotso et al. 2002).

D. Yamagishi · H. Akamatsu[1] · H. Otani · M. Kodama (✉)
Laboratory of Plant Pathology, Faculty of Agriculture, Tottori University, 4-101 Koyama-Minami, Tottori 680-8553, Japan
Tel. +81-857-31-5364; Fax +81-857-31-5356
e-mail: mk@muses.tottori-u.ac.jp

Present address:
[1]Department of Plant Pathology, Washington State University, Pullman, WA, USA

The AAL-toxins and fumonisins are structurally related and were originally isolated from the tomato pathotype of *Alternaria alternata* (synonym *Alternaria alternata* f. sp. *lycopersici*, synonym *Alternaria arborescens*) and from *Fusarium verticillioides* (Sacc.) Nirenberg (synonym *Fusarium moniliforme*, teleomorph *Gibberella moniliformis*, synonym *Gibberella fujikuroi* mating population A), respectively (Gilchrist and Grogan 1976; Kohmoto et al. 1989; Bezuidenhout et al. 1988; Peever et al. 2004). AAL-toxins and fumonisins are sphinganine-analog mycotoxins (SAMs), which are toxic to some plant species and mammalian cells (Gilchrist et al. 1995). They cause apoptosis in susceptible tomato cells and mammalian cells by inhibiting ceramide biosynthesis (Gilchrist et al. 1995; Wang et al. 1996; Spassieva et al. 2002). In the tomato pathotype of *A. alternata*–tomato interactions, a major factor in pathogenicity is the production of host-specific AAL-toxins that are capable of inducing cell death only in susceptible cultivars (Akamatsu et al. 1997; Brandwagt et al. 2000). There are also reports indicating that the tomato pathotype of *A. alternata* (*A. alternata* f. sp. *lycopersici*) can produce fumonisins in culture (Chen et al. 1992; Mirocha et al. 1992, 1996). However, in *F. verticillioides*–maize pathogenic interactions, at least, fumonisins are not a major factor for pathogenicity in the field (Desjardins et al. 2002). The role of fumonisins in pathogenicity therefore remains obscure, raising several questions: (1) Do the Japanese isolates of the tomato pathotype of *A. alternata* also produce fumonisins? (2) Do fumonisins also act as pathogenicity factors in the tomato pathotype for successful infection on susceptible tomato plants? (3) Do isolates of AAL-toxin-producing *A. alternata* and fumonisin-producing *Fusarium* spp. share genes for the SAMs production? To answer these questions, we analyzed the production of SAMs in *Alternaria* spp. and *Fusarium* spp. and examined infection-inducing activity of fumonisins against an *Alternaria* isolate on tomato. The results presented in this article support the possibility that AAL-toxin is sufficient for pathogenicity of the tomato pathotype of *A. alternata* even though fumonisin has toxigenic and infection-inducing activities on the susceptible tomato plants.

Table 1. *Alternaria* and *Fusarium* species and production of AAL-toxin and fumonisin

		Strain	AAL-toxin T_A[a]	Fumonisin B_1
Alternaria alternata	Tomato pathotype	As-27	38.1 ± 7.0	ND
		H6	2.3 ± 0.3	ND
		AL4	525.6 ± 66.0	ND
		VU2001	2.98 ± 0.7	ND
		O-229	0.8 ± 0.6	ND
		EGS 39-128	18.5 ± 2.3	ND
	Japanese pear pathotype	O-276	ND	ND
	Strawberry pathotype	NAF8	ND	ND
	Nonpathogen	O-94	ND	ND
Gibberella fujikuroi	MP-A	A4249	ND	81.8 ± 15.7
	MP-C	F-113	ND	20.6 ± 3.5
	MP-C	CTF 129-2	ND	ND
	MP-C	C1993	ND	ND
	MP-D	FP-1720	ND	162.2 ± 20.1
	MP-D	FP-1727	ND	1190.9 ± 227.6
Fusarium graminearum		F-112	ND	ND
F. sporotrichioides		F-81	ND	ND
F. oxysporum	f. sp. *lycopersici*	880621a-1	ND	ND
		MAFF 305121	ND	ND
		F-1-1	ND	ND
		Tomino1-c	ND	ND
		NBRC 6531	ND	ND
		MAFF 103038	ND	ND
	f. sp. *melonis*	F-116	ND	463.9 ± 134.7
		F-119	ND	ND
	f. sp. *batatas*	O-17	ND	ND
	f. sp. *conglutinans*	Cong:1-1	ND	ND
	f. sp. *cucumerinum*	Rif-1	ND	ND
	f. sp. *melongenae*	MAFF 103051	ND	ND
	f. sp. *apii*	860926a	ND	ND
	f. sp. *niveum*	MAFF 305608	ND	ND

ND, not detected
[a] Amounts (μg/g) of AAL-toxin T_A and fumonisin B_1 were determined by HPLC with *o*-phthalaldehyde derivatization as described in Fig. 1

Alternaria, *Gibberella*, and *Fusarium* species used in this study are listed in Table 1. Some of the *Fusarium* species have been reported to produce fumonisins (Rheeder et al. 2002). These include *F. verticillioides* (Sacc.) Nirenberg (previously known as *F. moniliforme* Sheldon), mating population A (MP-A), and *Fusarium fujikuroi* Nirenberg MP-C, *Fusarium proliferatum* (Matsushima) Nirenberg MP-D. High levels of FB_1, FB_2, and FB_3 production were observed in *F. verticillioides* and *F. proliferatum*, while trace amounts of FB_1 was produced by *F. fujikuroi* (Rheeder et al. 2002). Production of fumonisins by *Fusarium oxysporum* f. sp. *melonis* has not been reported yet, although *F. oxysporum* var. *redolens* is known to produce FB_1, FB_2, and FB_3 (Rheeder et al. 2002). Production of low levels of fumonisins by some isolates of the tomato pathotype of *A. alternata* has been reported (Chen et al. 1992; Mirocha et al. 1992, 1996). There has been no report until now on the contamination of crops by fumonisins produced by *Alternaria* species. It is important to evaluate fumonisin production by *Alternaria* species, because *Alternaria* is one of the most ubiquitous fungal groups in the world with a wide geographical distribution and frequent occurrence on many important crops.

The production of AAL-toxin and fumonisin was assessed in cultures grown on autoclaved rice. In brief, 2 g of polished rice in a test tube was moistened for 1 h with 0.9 ml of distilled water and autoclaved at 121°C for 60 min. Rice media were inoculated with mycelium agar plugs of *Alternaria* and *Fusarium* species prepared from 7-day-old cultures grown on potato-dextrose agar in petri dishes. The inoculated rice media was incubated at 26°C for 15 days. Samples of each fungus were dried in a forced-air draft oven (Sanyo, Tokyo, Japan) at 55°C for 48 h. The dried cultures were crushed with a mortar and pestle and extracted with 1 ml of $CH_3CN:H_2O$ (1:1, v/v) for 1 h. The extracts were centrifuged at 13000 rpm for 10 min, and the supernatants were cleaned on a Sep-Pak C18 cartridge column (Waters, Milford, MA, USA) with $CH_3CN:H_2O$ (1:1, v/v) as a solvent.

The levels of AAL-toxins and fumonisins were determined directly from samples of the extracts using high performance liquid chromatography (HPLC) with precolumn derivatization of the toxins with *o*-phthalaldehyde (OPA) as described previously (Kodama et al. 1995) with slight modifications. The OPA reagent was prepared by dissolving 50 mg of OPA in 1.25 ml of methanol and adding 50 μl of 2-mercaptoethanol (Wako, Osaka, Japan) and 11.2 ml of 0.1 M sodium borate buffer (pH 9.5). The sample (25 μl) was mixed with reagent (125 μl) and incubated for 2 min at room temperature, and then injected onto the HPLC column. HPLC analysis of the derivatives was performed using a Hitachi HPLC system equipped with a F-1050 fluorescence

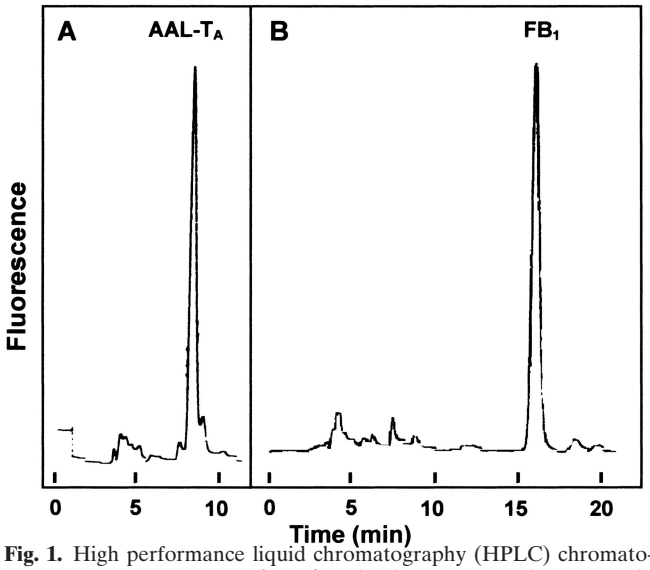

Fig. 1. High performance liquid chromatography (HPLC) chromatograms of *o*-phthalaldehyde (OPA) derivatives of authentic AAL-toxin T_A (**A**) and fumonisin B_1 (**B**). HPLC was carried out using a μBondasphere C18 column (3.9 × 150 mm) with a μBondapak C18 Guard Pak using methanol/0.1 M sodium dihydrogenphosphate buffer (pH 3.3) (75:25, v/v) at a flow rate of 0.7 ml/min

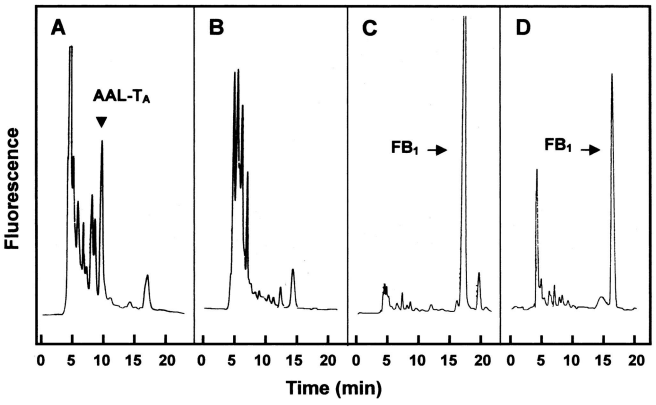

Fig. 2. HPLC chromatograms of culture filtrates of two *Alternaria* isolates [As-27 (**A**) and O-94 (**B**)] and two *Fusarium* isolates [A4249 (**C**) and F-116 (**D**)]. Isolates of *Alternaria* and *Fusarium* species were grown on rice for 15 days. Samples of each fungus were dried and extracted with $CH_3CN:H_2O$ (1:1, v/v). The extracts were cleaned by a Sep-Pak C18 cartridge column with $CH_3CN:H_2O$ (7:3, v/v) as solvent. OPA derivatization and HPLC analysis are described in Fig. 1. Peaks for AAL-toxin T_A (AAL-T_A) and fumonisin B_1 (FB_1) are indicated by *arrows*

spectrophotometer, L-6000 pump, L-5000 LC controller, and D-2500 chromato-integrator (Hitachi, Tokyo, Japan). The sample (10 μl) was injected and chromatographed on a Waters μBondasphere C18 column (3.9 × 150 mm) with a μBondapak C18 Guard Pak using methanol/0.1 M sodium dihydrogenphosphate buffer (pH 3.3) (75:25, v/v) at a flow rate of 0.7 ml/min. The excitation and emission wavelengths were 335 nm and 440 nm, respectively. Purified AAL-toxins (Kodama et al. 1995) and fumonisin B_1 (Sigma-Aldrich, St. Louis, MO, USA) were used as standards.

The chromatogram obtained from the OPA derivatives of the standard AAL-toxin T_A and fumonisin B_1 is shown in Fig. 1. Two well-resolved peaks were seen at retention times of 9.35 min (AAL-toxin T_A) and 17.58 min (fumonisin B_1) on the chromatogram. The detection limit was of the order of 10 ng. Chromatograms of two *Alternaria* isolates (As-27 and O-94) and two *Fusarium* isolates (A4249 and F-116) are shown in Fig. 2. The baseline separation of the two toxins produced in the cultures by the pathogens was achieved using this method (Fig. 2).

All strains of the tomato pathotype produced AAL-toxin, but no fumonisin (Table 1). Nonpathogenic *A. alternata* and several non-tomato pathotypes were shown to not produce AAL-toxin or fumonisin (Table 1). On the other hand, strains of *F. verticillioides*, *F. fujikuroi*, and *F. proliferatum* all except CTF 129-2 and C1993 produced fumonisin, but none of them produced AAL-toxin (Table 1). This is the first report of fumonisin production by an isolate of *F. oxysporum* f. sp. *melonis*.

To further explore the potential ability for SAMs production by the isolates of *Alternaria* and *Fusarium* species, a polymerase chain reaction (PCR)-based method to specifically detect genes for biosynthesis of AAL-toxin or fumonisin was used. Genomic DNAs of the isolates of *Alternaria* and *Fusarium* species were extracted as described previously (Akamatsu et al. 1997), and nucleic acid manipulations followed standard procedures (Sambrook et al. 1989). To detect genes for AAL-toxin (*ALT1*) (Akamatsu et al. 2003) and fumonisin (*FUM1*) (Proctor et al. 1999), primer pairs for *ALT1* (Al1, 5′-CGAATCGCATCAGC TACGAA-3′; Al2, 5′-GATTGCACAGTGTGTATGCC-3′) and *FUM1* (Fm1, 5′-GACACCTCCTTCTTCTC CATG-3′; Fm2, 5′-GTGCCGGTTCCGTGTGCTTC-3′) were used in PCR experiments with genomic DNAs of the isolates, respectively. PCR was performed in a GeneAmp PCR system 9600 (Applied Biosystems, Foster City, CA, USA) with an initial step of 5 min at 95°C followed by 30 cycles of 15 s at 95°C, 30 s at 65°C, 1.5 min at 72°C, and a final step of 5 min at 72°C. A PCR product of the expected size (1.2 kb) for *ALT1* was only amplified from the tomato pathotype isolates (Fig. 3). No other products were identified from nonpathogenic *A. alternata* O-94 or any *Fusarium* species. A PCR product of the expected size (0.7 kb) for *FUM1* was detected in *F. verticillioides* (*G. fujikuroi* MP A) (A4249), *F. fujikuroi* (*G. fujikuroi* MP C) (F-113 and CTF 129-2), *F. proliferatum* (*G. fujikuroi* MP D) (FP-1727), and *F. oxysporum* f. sp. *melonis* (F-116) (Fig. 3). The results indicate that the gene for AAL-toxin biosynthesis is only distributed in the tomato pathotype isolates examined here. The *Alternata* isolates, however, do not possess the gene for fumonisin biosynthesis. Fumonisin productivity in *F. oxysporum* f. sp. *melonis* was also supported by this gene-testing method. *Gibberella fujikuroi* MP C isolates CTF 129-2 and C1993 amplified a 0.7-kb product specific for *FUM1* despite the lack of detectable fumonisin B_1 production in the isolates (Table 1, Fig. 3). The reason for this inconsistency may be attributed to the possible defect of genes in *FUM* cluster other than *FUM1* (Proctor et al. 2003).

The biological activities of AAL-toxin and fumonisin on the susceptible tomato were examined with a leaf necrosis bioassay. Different concentrations of the toxin solutions were added to wells of a multiplate (24 wells) (Corning, Corning, NY, USA). Leaf discs (15 mm in diameter) were cut from the susceptible cultivar Aichi-first and placed in wells of the plates and incubated at 25°C for 4 days under fluorescent light. The minimum concentrations for necrosis formation on the leaves of the highly susceptible cultivar Aichi-first by AAL-toxin and fumonisin were 0.01 and 0.05 µg/ml, respectively (Fig. 4). Therefore, fumonisin is about five times less active than AAL-toxin. On the other hand, AAL-toxin was reported to be slightly less toxic against animal cells than fumonisin (Mirocha et al. 1992). The similarity of the AAL-toxin and fumonisin activities fits with the knowledge that they both inhibit ceramide synthesis and kill the host cells by inducing programmed cell death (Markham and Hille 2001; Spassieva et al. 2002; Brandwagt et al. 2000).

In addition to the direct toxicities of the SAMs, infection-inducing activity of the toxins was also examined. Many lines of evidence have supported the concept that the key role of HSTs in initial colonization is not to kill host cells but to induce accessibility of the cells to toxin-producing pathogens (Kohmoto et al. 1989). From this viewpoint, infection-inducing activity of fumonisin was compared with that of AAL-toxin on susceptible tomato leaves. Spores of nonpathogenic *A. alternata* O-94 and the tomato pathotype As-27 were prepared as previously described (Otani et al. 1998). Spores of O-94 were suspended in either AAL-toxin

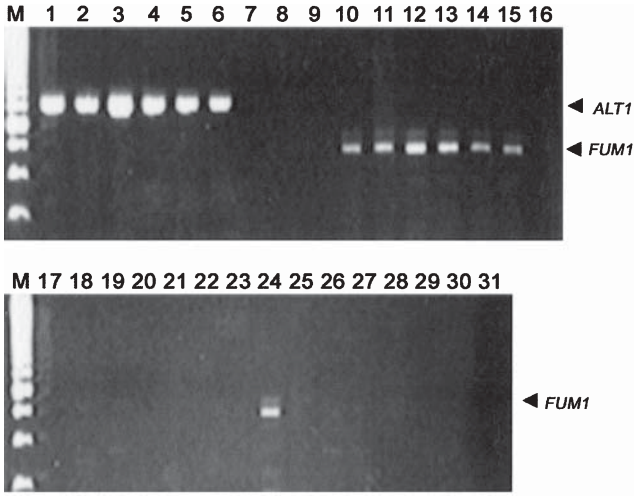

Fig. 3. Distribution of genes for AAL-toxin (*ALT1*) and fumonisin (*FUM1*) biosynthesis in *Alternaria* and *Fusarium* species listed in Table 1. Polymerase chain reaction (PCR) primers Al1/Al2 and Fm1/Fm2 detected *ALT1* (1.2 kb) and *FUM1* (0.7 kb) genes, respectively. Size markers are 200 bp ladder (*M*). *1*, As-27; *2*, H6; *3*, AL4; *4*, VU2001; *5*, O-229; *6*, EGS 39–128; *7*, O-276; *8*, NAF 8; *9*, O-94; *10*, A4249; *11*, F-113; *12*, CTF 129-2; *13*, C1993; *14*, FP-1720; *15*, FP-1727; *16*, F-112; *17*, F-81; *18*, 880621a-1; *19*, MAFF 305121; *20*, F-1-1; *21*, Tomino1-c; *22*, NBRC 6531; *23*, MAFF 103038; *24*, F-116; *25*, F-119; *26*, O-17; *27*, Cong:1-1; *28*, Rif-1; *29*, MAFF 103051; *30*, 860926a; *31*, MAFF 305608

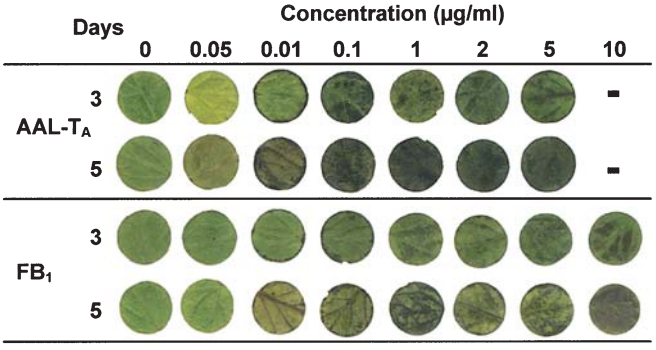

Fig. 4. Leaf necrosis bioassay of AAL-toxin T_A and fumonisin B_1 with susceptible tomato leaves. Leaf discs of the susceptible cultivar Aichi-first were incubated with different concentrations of AAL-toxin T_A or fumonisin B_1 at 25°C for 3 or 5 days

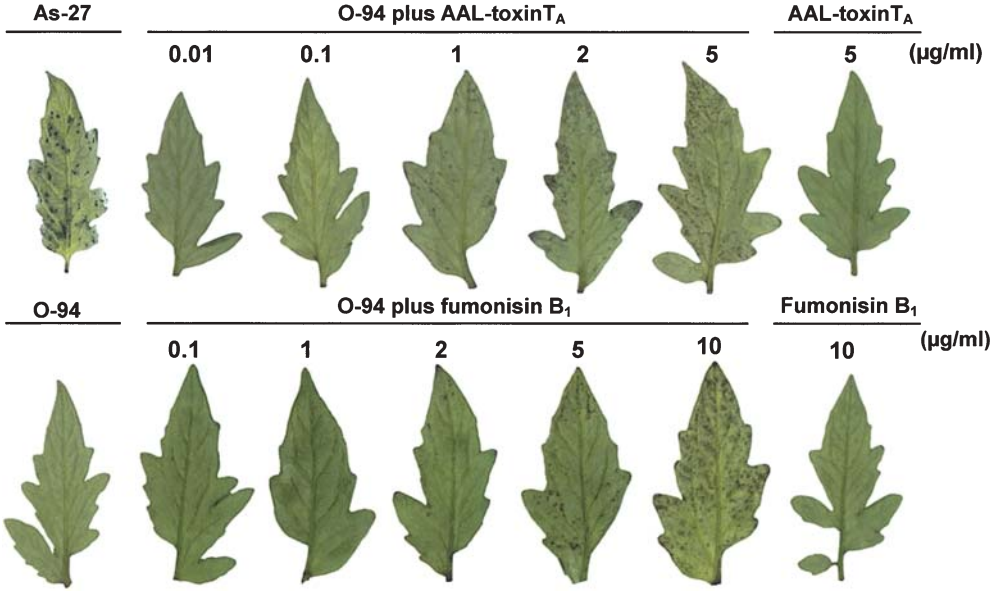

Fig. 5. Effects of AAL-toxin and fumonisin on infection by nonpathogenic *Alternaria alternata* (O-94) on susceptible tomato leaves. Spores of O-94 were suspended in AAL-toxin T_A or fumonisin B_1 solutions at concentrations of 0.01–5 µg/ml or 0.1–10 µg/ml, respectively, and sprayed on the lower surface of susceptible tomato cultivar Aichi-first leaves. Control leaves were sprayed with spores of O-94 and As-27 suspended in distilled water at the same density (10^5 spores/ml). The leaves were incubated in a moist chamber at 25°C for 24 h

T_A or fumonisin B_1 solutions at concentrations of 0.01–5 μg/ml or 0.1–10 μg/ml, respectively. The spore suspensions were sprayed on the lower surface of susceptible tomato cultivar Aichi-first leaves. As controls, leaves were sprayed with spores of O-94 and As-27 suspended in distilled water at the same density. The leaves were incubated in a moist chamber at 25°C for 3 days, then lesion development on the leaves was evaluated. When spores of O-94 combined with 1 μg/ml of AAL-toxin T_A or 5 μg/ml of fumonisin B_1 were sprayed on the leaves, the formation of necrotic lesions was similar to the control inoculation with pathogenic As-27 (Fig. 5). To confirm that the necrotic lesions were a result of infection rather than a direct effect of the toxins, solutions of up to 5 μg/ml AAL-toxin T_A or 10 μg/ml fumonisin B_1 were sprayed on uninjured leaves. No visible necrosis was detected (Fig. 5). The results indicate that fumonisin can induce infection of nonpathogenic *A. alternata* in a similar manner to that of AAL-toxin, although the effective concentration required is higher than that of AAL-toxin.

Our results suggest that fumonisin should also be a factor for pathogenicity/virulence for the tomato pathotype of *A. alternata* because it has almost the same biological effects as AAL-toxin, although it is less active. However, we detected no fumonisin in the tomato pathotype isolates (Table 1). It is perhaps not surprising that fumonisin acts similarly to AAL-toxin, given their common structures and known activities. Taken together, AAL-toxin production alone is sufficient for the tomato pathotype of *A. alternata* to mount a successful infection on susceptible tomato plants, although previous reports also suggested a possible role for fumonisin production in the pathogenesis of the tomato pathotype of *A. alternata* (Chen et al. 1992; Mirocha et al. 1992, 1996).

Acknowledgments This work was partly supported by a Grant-in-Aid for Scientific Research (C) from the Japan Society for the Promotion of Sciences (JSPS). We are grateful to Drs. R. D. Johnson (AgResearch Limited) and T. Arie (Tokyo University of Agriculture and Technology) for valuable suggestions. We thank Drs. T. Arie, T. Yoshida (National Institute of Agro-Environmental Sciences), Y. Hosobuchi (Sakata Seed), S. Ouchi, and A. Moretti (Instituto Science dell Produzioni Alimentari C.N.R) for providing fungal isolates.

References

Akamatsu H, Itoh Y, Kodama M, Otani H, Kohmoto K (1997) AAL-toxin deficient mutants of *Alternaria alternata* tomato pathotype by restriction enzyme-mediated integration. Phytopathology 87:967–972

Akamatsu H, Otani H, Kodama M (2003) Characterization of a gene cluster for host-specific AAL-toxin biosynthesis in the tomato pathotype of *Alternaria alternata*. Abstracts of the 22nd Fungal Genetics Conference, Asilomar, CA, USA, 2003, p 121

Bezuidenhout SC, Gelderblom WCA, Gorst-Allman CP, Horak RM, Marasas WFO, Spiteller G, Vleggaar R (1988) Structure elucidation the fumonisins, mycotoxins from *Fusarium moniliforme*. J Chem Soc Chem Commun 1988:743–745

Brandwagt BF, Mesbah LA, Takken FLW, Laurent PL, Kneppers TJA, Hille J, Nijkamp HJJ (2000) A longevity assurance gene homolog of tomato mediates resistance to *Alternaria alternata* f. sp. *lycopersici* toxins and fumonisin B1. Proc Natl Acad Sci USA 97:4961–4966

Chen J, Mirocha CJ, Xie W, Hogge L, Oison D (1992) Producion of the mycotoxin fumonisin B_1 by *Alternaria alternata* f. sp. *lycopersici* and AAL-toxin. Phytopathology 77:80–82

Desjardins AE, Munkvold GP, Plattner RD, Proctor RH (2002) *FUM1*—a gene required for fumonisin biosynthesis but not for maize ear rot and ear infection by *Gibberella moniliformis* in field tests. Mol Plant Microbe Interact 15:1157–1164

Fotso J, Leslie JF, Smith JS (2002) Production of beauvericin, moniliformin, fusaproliferin, and fumonisin B1, B2, and B3 by fifteen ex-type strains of *Fusarium* species. Appl Environ Microbiol 68:5195–5197

Gilchrist DG, Grogan RG (1976) Production and nature of a host-specific toxin from *Alternaria alternata* f. sp. *lycopersici*. Phytopathology 66:165–171

Gilchrist DG, Wang H, Bostock RM (1995) Sphingosine-related mycotoxins in plant and animal disease. Can J Bot 73:S459–S467

Kodama M, Otani H, Kohmoto K (1995) A rapid and sensitive procedure for the quantitative detection of AL-toxin by fluorescence derivatization and separation by high performance liquid chromatography. Ann Phytopathol Soc Jpn 61:477–480

Kohmoto K, Otani H, Kodama M, Nishimura S (1989) Host recognition: can accessibility to fungal invasion be induced by host-specific toxins without necessitating necrotic cell death? In: Graniti A, Durbin RD, Ballio A (eds) Phytotoxins and plant pathogenesis. Springer, Berlin Heidelberg New York, pp 249–265

Markham JE, Hille J (2001) Host-selective toxins as agents of cell death in plant–fungus interactions. Mol Plant Pathol 2:229–239

Mirocha CJ, Gilchrist DG, Shier WT, Abbas HK, Wen Y, Vesonder RF (1992) AAL toxins, fumonisins (biology and chemistry) and host-specificity concepts. Mycopathologia 117:47–56

Mirocha CJ, Chen J, Xie W, Xu Y, Abbas HK, Hogge LR (1996) Biosynthesis of fumonisin and AAL derivatives by *Alternaria* and *Fusarium* in laboratory culture. In: Jackson LS, DeVries JW, Bullerman LB (eds) Fumonisins in food. Plenum, New York, pp 213–224

Otani H, Kohmoto K, Kodama M (1995) *Alternaria* toxins and their effects on host plant. Can J Bot 73:S453–S458

Otani H, Kohnobe A, Kodama M, Kohmoto K (1998) Production of a host-specific toxin by germinating spores of *Alternaria brassicicola*. Physiol Mol Plant Pathol 52:285–295

Peever TL, Su G, Carpenter-Boggs L, Timmer LW (2004) Molecular systematics of citrus-associated *Alternaria* species. Mycologia 96:119–134

Proctor RH, Desjardins AE, Plattner RD, Hohn TM (1999) A polyketide synthase gene required for biosynthesis of fumonisin mycotoxins in *Gibberella fujikuroi* mating population A. Fungal Genet Biol 27:100–112

Proctor RH, Brown DW, Plattner RD, Desjardins AE (2003) Co-expression of 15 contiguous genes delineates a fumonisin biosynthetic gene cluster in *Gibberella moniliformis*. Fungal Genet Biol 38:237–249

Rheeder JP, Marasas WFO, Vismer HF (2002) Production of fumonisin analogs by *Fusarium* species. Appl Environ Microbiol 68:2101–2105

Sambrook J, Fritsch EF, Maniatis TA (1989) Molecular cloning: a laboratory manual, 2nd edn. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

Spassieva SD, Markham JE, Hille J (2002) The plant disease resistance gene *Asc-1* prevents disruption of sphingolipid metabolism during AAL-toxin-induced programmed cell death. Plant J 32:561–572

Wang H, Li J, Bostock RM, Gilchrist DG (1996) Apoptosis: a functional paradigm for programmed plant cell death induced by a host-selective phytotoxin and invoked during development. Plant Cell 8:375–391